interaction between RNA and inhibitory molecules. In some cases, even harsh conditions do not stop the interaction. In other cases, the harsh conditions interfere with downstream applications of the RNA. Accordingly, a method is needed which neutralizes or mitigates the interaction of inhibitory molecules to RNA, but does not interfere with the function and analysis of RNA.

Please amend the paragraph beginning at line 24, page 1 to read as follows:

The invention describes the addition of $(NH_4)_2SO_4$ to an environment containing RNA. The final concentration is below 20 g/100 ml (1.51 M). The addition of $(NH_4)_2SO_4$ to the environment neutralizes the inhibitory effects of agents that bind to, or cleave, RNA. Such agents include cationic detergents (*e.g.*, CATRIMOX and cetyltrimethylammonium bromide (CTAB). *See, e.g.*, European Patent Application EP 1031626 A1), proteins, ethidium bromide, SYBRGREEN® dye, polyamines (*e.g.*, spermine, spermidine, putresceine, etc.), charged polysaccharides, glycoproteins, nucleophiles, bases etc. In the presence of $(NH_4)_2SO_4$, the inhibitory or cleaving properties of agents that bind to RNA is reduced or eliminated.

Please amend the paragraph beginning at line 23, page 2 to read as follows:

In any of the aforesaid aspects, the composition may further comprise a contaminant selected from RNA binding agents. In any of the aforesaid aspects, the composition may further comprise a polyamine as a contaminant, where spermine, spermidine, and putresceine are exemplary polyamine contaminants. In any of the aforesaid aspects, the composition may further comprise a cationic detergent as a contaminant. In any of the aforesaid aspects, the composition may further comprise a nucleic acid dye as a contaminant, where ethidium bromide and SYBRGREEN® dye are exemplary nucleic acid dye contaminants. In any of the aforesaid aspects, the composition may further comprise actinomycin as a contaminant. In any of the aforesaid aspects, the composition may further comprise a charged polysaccharide as a contaminant. In any of the aforesaid aspects, the composition may further comprise glycoprotein as a contaminant. In any of the aforesaid aspects, the composition may further comprise a nucleophile as a contaminant. In additional aspects, the present invention provides that the composition to which ammonium sulfate is added may contain any two or more of the specifically enumerated contaminants, *i.e.*, any two or more (*e.g.*, three, four) of RNA binding

agent, polyamine, cationic detergent, nucleic acid dye, actinomycin, charged polysaccharide, glycoprotein, and nucleophile.

Please amend the paragraph beginning at line 21, page 3 to read as follows:

Figure 4 shows improvement in the performance of TaqMan RT-PCR by the addition of ammonium sulfate when cationic detergents were present in the reaction mixture. The threshold-cycle of each bar reflects the average of six independent values.

Please amend the paragraph beginning at line 24, page 3 to read as follows:

Figure 5 shows stability of the neutralization effect during time. RNA prepared in the absence of any cationic detergent with elution in water (C), in the presence of a cationic detergent with elution in water (B), or in the presence of a cationic detergent with elution in 10 mM $(NH_4)_2SO_4$ (A) was used in TaqMan RT-PCR reactions for amplifying GAPDH (glyceraldehydes-3-phosphate dehydrogenase). Each bar in this figure represents the average of the analyses of three independent blood donors.

Please amend the paragraph beginning at line 1, page 4 to read as follows:

Figure 6 shows stability of the neutralization effect during time. RNA prepared in the absence of any cationic detergent with elution in water (C), in the presence of a cationic detergent with elution in water (B), or in the presence of a cationic detergent with elution in 10 mM $(NH_4)_2SO_4$ (A) was used in TaqMan RT-PCR reactions for amplifying TNFalpha (Tumor Necrosis Factor alpha). Each bar in this figure represents the average of the analyses of three independent blood donors.

Please amend the paragraph beginning at line 5, page 4 to read as follows:

Figure 7 shows inhibitory effects of spermine during reverse transcription reactions. The concentrations of spermine in lanes 1-5 are: 0 mM, 0.125 mM, 0.25 mM, 0.5 mM, and 1 mM, respectively.

Please amend the paragraph beginning at line 11, page 4 to read as follows:

Figure 9 shows inhibitory effects of SYBRGREEN® dye during reverse transcription reaction. Final concentrations of SYBRGREEN® dye in the reverse transcription reaction mixture are indicated. PCR was performed to quantify cDNA synthesis.

Please amend the paragraph beginning at line 14, page 4 to read as follows:

Figure 10 shows electrophoretic analysis of RT-PCR products from an RNA sample that contains SYBRGREEN® dye, but no ammonium sulfate (lanes 2), an RNA sample that contains both SYBRGREEN® dye and ammonium sulfate (lanes 1), and an RNA sample that contains neither SYBRGREEN® dye nor ammonium sulfate as a control (Lane 3).

Please amend the paragraph beginning at line 24, page 4 to read as follows:

Figure 14 shows relative activities of a reverse transcriptase using RNAs dissolved in solutions of different $(NH_4)_2SO_4$ concentrations as templates. The bars with three different shades represent results from three different lots of reverse transcriptases.

Please amend the paragraph beginning at line 4, page 5 to read as follows:

Ribonucleic acid (RNA) is a substance synthesized biologically and synthetically. RNA serves many functions as information molecule, reaction substrate, reaction catalyst, recognition element, structural element, etc. For most analysis methods and functions concerning RNA, the purity of RNA is important. For instance, other molecules present in an RNA sample, or in a reaction mixture in which RNA molecules participate, may inhibit the analysis or function of the RNA molecules or destroy the structure of the RNA molecules. Thus, it is important to reduce or eliminate the inhibitory or destructive effects of such molecules. Moreover, stable secondary structures of RNA may also interfere with RNA function or analysis. The present invention features the addition of ammonium sulfate to an RNA solution to eliminate or reduce the inhibitory or destructive effects of certain other molecules (*e.g.*, those that bind to the RNA) in the solution that interferes with the use of RNA as information molecule, reaction substrate, reaction catalyst, recognition element, structural element etc. Furthermore, $(NH_4)_2SO_4$ solves secondary structures of RNA to make RNA more accessible to reactions and analysis

Please amend the paragraph beginning at line 19, page 5 to read as follows:

The present invention is directed to the addition of $(NH_4)_2SO_4$ to a composition containing RNA. In one aspect, the final concentration of the $(NH_4)_2SO_4$ is below 20 g/100 mL (1.51 M). The addition of $(NH_4)_2SO_4$ to the environment neutralizes the inhibitory effects of agents that interferes with RNA function and/or analysis, such as those that bind to, or cleave, the RNA. Such agents include cationic detergents (*e.g.*, CATRIMOX and cetyltrimethylammonium bromide (CTAB). *See, e.g.*, European Patent Application EP 1031626 A1), proteins, ethidium bromide, SYBRGREEN® dye, polyamines (*e.g.*, spermine, spermidine, putresceine etc.), charged polysaccharides, glycoproteins, nucleophiles, bases, etc.

A12

Please amend the paragraph beginning at line 14, page 11 to read as follows:

RNA is a substance synthesized biologically and synthetically. RNA serves many functions as information molecule, reaction substrate, reaction catalyst, recognition element, structural element, etc. For most analysis methods and functions concerning RNA, the purity of RNA is important. For instance, other molecules present in an RNA sample, or in a reaction mixture that RNA molecules participate, may inhibit the analysis or function of the RNA molecules or destroy the structure of the RNA molecules. Thus, it is important to reduce or eliminate the inhibitory or destructive effects of such molecules. Moreover, stable secondary structures of RNA may also interfere with RNA function or analysis. The present invention features the addition of ammonium sulfate to an RNA solution to eliminate or reduce the inhibitory or destructive effects of certain other molecules (*e.g.*, those that bind to the RNA) in the solution that interferes with the use of RNA as information molecule, reaction substrate, reaction catalyst, recognition element, structural element, etc. Furthermore $(NH_4)_2SO_4$ solves secondary structures of RNA to make RNA more accessible to reactions and analysis.

A13

Please amend the paragraph beginning at line 1, page 12 to read as follows:

The present invention is directed to the addition of $(NH_4)_2SO_4$ to a composition containing RNA. In one aspect, the final concentration of the $(NH_4)_2SO_4$ is below 20 g/100 mL (1.51 M). The addition of $(NH_4)_2SO_4$ to the environment neutralizes the inhibitory effects of agents that interferes with RNA function and/or analysis, such as those that bind to, or cleave,

A14

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the RNA. Such agents include cationic detergents (*e.g.*, CATRIMOX and cetyltrimethylammonium bromide (CTAB). *See, e.g.*, European Patent Application EP 1031626 A1), proteins, ethidium bromide, SYBRGREEN® dye, polyamines (*e.g.*, spermine, spermidine, putresceine etc.), charged polysaccharides, glycoproteins, nucleophiles, bases, etc.

Please amend the paragraph beginning at line 22, page 14 to read as follows:

Human blood RNA was prepared with a cationic detergent or with a classical method in the absence of any cationic detergent. The RNA was eluted with water or with 10 mM $(NH_4)_2SO_4$. The eluate containing 10 mM $(NH_4)_2SO_4$ was denaturated at 65°C for 5 minutes and cooled on ice. An aliquot of each eluate was transferred to a single-tube TaqMan RT-PCR mixture to amplify a GAPDH fragment. Ingredients of the above reaction were provided by Applied Biosystem (PDAR (Pre-Developed Assay Reagents) GAPDH).

Please amend Example 8 at page 17 to read as follows:

EXAMPLE 8

SYBRGREEN® DYE INHIBITS RT-PCR REACTIONS

This example shows that SYBRGREEN® dye inhibits RT-PCR reactions. As shown in Figure 9, SYBRGREEN® dye at a final concentration of 100x resulted in a total loss of cDNA synthesis.

Experimental set-up:

Reverse transcription reactions containing 0x, 0.001x, 0.01x, 0.1x, 1x, 10x and 100x SYBRGREEN® dye were performed. In order to quantify the generated cDNA after RT-reaction, 2 µl of the RT reaction was transferred to a 20 µl PCR mixture. PCR products were analyzed by gel-electrophoresis.

Please amend Example 9 at pages 17 and 18 to read as follows:

EXAMPLE 9

$(NH_4)_2SO_4$ MITIGATES THE INHIBITORY EFFECTS OF SYBRGREEN® DYE ON RT-PCR

a17

Reverse transcriptase is not able to displace SYBRGREEN® dye binding to RNA. Thus, RNA is masked and cannot be analyzed quantitatively. Due to the binding of SYBRGREEN® dye to RNA, only signal with very low intensities were obtained during rRT-PCR. A denaturation step (5 minutes at 65°C, shock cool on ice) of RNA alone did not solve the complex of RNA and SYBRGREEN® dye. The addition of $(NH_4)_2SO_4$ to the final concentration of 5 mM followed by denaturation of the sample for 5 minutes at 65°C with shock cool on ice significantly increased the RT-PCR signal (Figure 10).

Experimental set-up:

Total RNA containing SYBRGREEN® dye was dissolved in 2 µl water (lanes 2) or in 2 µl of a 5 mM $(NH_4)_2SO_4$ solution (lanes 1). In lanes 3, total RNA without SYBRGREEN® dye was dissolved in 2 µl of a 5 mM $(NH_4)_2SO_4$ solution. The solution was denaturated at 65°C for 5 minutes and cooled on ice. The whole solution was transferred to a 20 µl RT reaction mixture and the RT reaction was performed at 37°C. After the RT reaction was finished, 2 µl of the RT reaction mixture was transferred to a 20 µl PCR mixture. The resulting PCR products were analyzed by gel-electrophoresis.

In the Claims:

Please amend claims 1-14 to read as follows:

a18

1. (Amended) A method to neutralize the inhibitory or destructive effect of an agent on the function or analysis of RNA isolated from a natural source or artificially synthesized, wherein the agent binds to, or cleaves, said RNA, comprising adding ammonium sulfate to a composition comprising said RNA and said agent, where the final concentration of ammonium sulfate in the composition is below 20 g / 100 mL, and whereby the inhibitory or destructive effect of said agent is neutralized.

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